

The underlying neurobiology of bipolar disorder

HUSSEINI K. MANJI, JORGE A. QUIROZ, JENNIFER L. PAYNE, JASKARAN SINGH, BARBARA P. LOPES, JENILEE S. VIEGAS, CARLOS A. ZARATE

Laboratory of Molecular Pathophysiology, Mood and Anxiety Disorders Program, National Institute of Mental Health, 49 Convent Dr., Bethesda, MD 20892-4405, USA

Clinical studies over the past decades have attempted to uncover the biological factors mediating the pathophysiology of bipolar disorder (BD) utilizing a variety of biochemical and neuroendocrine strategies. Indeed, assessments of cerebrospinal fluid chemistry, neuroendocrine responses to pharmacological challenge, and neuroreceptor and transporter binding have demonstrated a number of abnormalities in the amine neurotransmitter systems in this disorder. However, recent studies have also implicated critical signal transduction pathways as being integral to the pathophysiology and treatment of BD, in addition to a growing body of data suggesting that impairments of neuroplasticity and cellular resilience may also underlie the pathophysiology of the disorder. It is thus noteworthy that mood stabilizers and antidepressants indirectly regulate a number of factors involved in cell survival pathways - including MAP kinases, CREB, BDNF and bcl-2 protein - and may thus bring about some of their delayed long-term beneficial effects via underappreciated neurotrophic effects.

Key words: Mania, norepinephrine, dopamine, acetylcholine, serotonin, glutamate, neuroplasticity, CREB, BDNF, ERK MAP kinase, bcl-2

Although genetic factors play a major, unquestionable role in the etiology of bipolar disorder (BD), the biochemical abnormalities underlying the predisposition to and the pathophysiology of BD remain to be fully elucidated. Early biologic theories regarding the pathophysiology of BD have focused upon various neurotransmitters, in particular the biogenic amines. In recent years, however, advances in our understanding of the cellular mechanisms underlying neuronal communication have focused research into the role of post-receptor sites. Indeed, the 'molecular medicine revolution' has resulted in a more complete understanding of the etiology and pathophysiology of a variety of medical disorders (1,2). However, in contrast to the progress that has been made in elucidating the etiology and/or pathophysiology of a variety of medical conditions, we have yet to identify the specific abnormal genes or proteins in BD. The behavioral and physiological manifestations of BD are complex and must account not only for the profound changes in mood, but also for the constellation of neurovegetative and psychomotor features. The pathophysiology is undoubtedly mediated by a network of interconnected limbic, striatal and fronto-cortical neurotransmitter neuronal circuits, and the interacting cholinergic, catecholaminergic and serotonergic neurotransmitter systems thus represent very attractive candidates. Thus, it is not surprising that clinical studies over the past 40 years have for the most part rested upon the conceptual foundation that monoamine signaling and hypothalamic-pituitary-adrenal (HPA) axis disruption are integral to the pathophysiology of both depression and mania (3,4).

A true understanding of the pathophysiology of BD must address its neurobiology at different physiological levels, i.e. molecular, cellular, systems, and behavioral. Abnormalities in gene expression undoubtedly underlie the neurobiology

of the disorder at the molecular level and this will become evident as we identify the susceptibility and protective genes for BD in the coming years. Once this has been accomplished, however, the even more difficult work must begin to examine the impact of the faulty expression of these gene-products (proteins) on integrated cell function. It is at these levels that some compelling protein candidates have been identified as the targets for the actions of mood stabilizing agents; however, the precise manner in which these candidate molecular and cellular targets may or may not relate to the faulty expression of susceptibility gene-products is yet to be determined. The task becomes even more daunting when one considers the possibility that a major component of the pathophysiology of BD may stem from discordant biological rhythms ranging from ultradian to infradian that ultimately drive the periodic recurrent nature of the disorder (5-7). The subsequent challenge for the basic and clinical neuroscientist will be the integration of these molecular/cellular changes to the systems and ultimately to the behavioral level wherein the clinical expression of BD becomes fully elaborated. However, considerable progress has been made in our understanding of this fascinating but devastating mental disorder. This article will focus upon recent data linking signaling abnormalities and impairments of neuroplasticity with the underlying neurobiology of BD. Space limitations necessitate the citing of review articles in many instances. A full reference list upon which this article is based is available upon request.

CLASSICAL MONOAMINERGIC NEUROTRANSMITTER AND NEUROENDOCRINE SYSTEMS

The stimulus for the study of the biogenic amines in patients with BD was provided by the discovery of effec-

tive pharmacologic treatments for depression and mania (3). In addition to these compelling pharmacological data, the biogenic amine neurotransmitter systems are distributed extensively in the limbic system, which is implicated in the regulation of sleep, appetite, arousal, sexual function, endocrine function, and emotional states such as fear and rage. The clinical picture of BD involves disruption of behavior, circadian rhythms, neurophysiology of sleep, neuroendocrine and biochemical regulation within the brain (3,8). These complex illness manifestations are undoubtedly mediated by a network of interconnected neurotransmitter pathways; the monoamine neurotransmitter systems are ideally placed to mediate such complex behavioral effects, and thus represent attractive candidate systems underlying the pathophysiology of BD (9).

Noradrenergic system

Despite methodological difficulties in assessing central nervous system (CNS) noradrenergic (NE) functions in humans, extensive investigation supports the presence of NE systems abnormalities in BD (3,10,11). Postmortem studies have shown an increased NE turnover in the cortical and thalamic areas of BD subjects (12,13), whereas in vivo studies have found plasma levels of NE and its major metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), to be lower in bipolar than unipolar depressed patients, and higher in bipolar patients when manic than when depressed (3,11). The same occurs with urinary MHPG levels, which are lower in bipolar depressed patients, while longitudinal studies show that MHPG excretion is higher in the manic compared to depressed state (3,4,10,11). Finally, in a consistent mode, cerebrospinal fluid (CSF) NE and MHPG are also reported to be higher in mania than in depression.

Other paradigms studying NE receptor function tend to suggest the possibility of an altered sensitivity of α_2 - and β_2 -adrenergic receptors in mood disorders (10,11). Genetics studies have also been carried out, showing that polymorphic variation of enzymes involved in amine metabolism (i.e. tyrosine hydroxylase, catechol-O-methyltransferase) could confer different susceptibility to develop bipolar symptomatology (14-16). However, although promising, these findings need to be replicated and subgroups of bipolar patients to whom these alterations may apply need to be identified.

Serotonergic system

There is a consistent body of data from CSF studies, neuroendocrine challenge studies, serotonin receptor and reuptake site binding studies, pharmacologic studies, and most recently, brain imaging studies supporting a role for alterations of serotonergic neurotransmission in major depressive episodes (3,17,18). Overall, investigators have reported reduced levels of 5-hydroxyindoleacetic acid (5-HIAA) in a

subgroup of patients, especially those with impulsivity, aggression and suicide attempts. In BD subjects, studies of CSF 5-HIAA in manic patients have generally produced variable and inconsistent results (3,19). Thus, baseline CSF 5-HIAA levels in manic patients, compared to non-depressed controls, have been reported as decreased in four studies, unchanged in nine studies, and increased in three studies; by contrast, most studies find no difference in the levels of CSF 5-HIAA between manic and depressed patients. Of the four studies that examined CSF 5-HIAA accumulation following administration of probenecid in manics, depressives and controls, two reported that both manic and depressed patients have diminished CSF 5-HIAA formation compared to controls, and one reported that manic patients have significantly lower CSF 5-HIAA accumulation than depressives and controls (3).

Studies have also reported decreased radioligand binding to the serotonin transporter (which takes up serotonin from the synaptic cleft) both in platelets and in the mid-brain of depressed patients (17,18). Most recently, an intriguing preliminary positron emission tomography (PET) study reported decreases in 5-hydroxytryptamine (5-HT)_{1A} receptor binding potential in raphe and hippocampus-amygdala of brain in depressed patients, in particular in bipolar depressives and in unipolar patients with bipolar relatives (20). One factor which may contribute to the reduction in 5-HT_{1A} receptor binding in depression is increased cortisol secretion (known to occur in many depressed patients, *vide infra*), since postsynaptic 5-HT_{1A} receptor mRNA expression is under tonic inhibition by corticosteroid receptor stimulation in some brain regions. The magnitude of the reduction in 5-HT_{1A} receptor density and mRNA levels induced by stress-induced glucocorticoid secretion in rodents is similar to that of the differences between depressed and healthy humans. For example, in rats, chronic unpredictable stress reduced 5-HT_{1A} receptor density an average of 22% across hippocampal subfields, similar to the 25% reduction in hippocampal 5-HT_{1A} receptor binding potential found in depression. Similarly, in tree shrews, chronic social subordination stress (for 28 days) decreased the density of 5-HT_{1A} receptors in the posterior cingulate, parietal cortex, prefrontal cortex (PFC), and hippocampus (by 11% to 34%), similar to the magnitude of reduced 5-HT_{1A} receptor binding potential found by Sargent et al (21) and Drevets et al (22) in these regions.

Neurotransmitter depletion models, specifically in this case tryptophan depletion to lower serotonin levels, permit a more direct strategy to clarify the involvement of serotonergic systems in mood disorders. Tryptophan depletion (achieved by the ingestion of preparations containing high levels of other aminoacids, but devoid of tryptophan) results in reversal of the response to certain antidepressant medications and recurrence of depression; however, depletion in healthy subjects without evidence of mental illness and in nonmedicated patients with depression does not consistently cause or intensify depression (23). These stud-

ies again substantiate the underlying complexity of neurobiologic systems not only in depression but by analogy in BD. With respect to BD, recent studies have investigated the effect of tryptophan depletion in lithium-treated euthymic patients and have generally found no recurrence of symptoms (24). Thus, although lithium has often been postulated to exert many of its beneficial effects via an enhancement of serotonergic function, the tryptophan depletion studies suggest that other mechanisms may be more important.

Most recently, investigators have explored the possibility that sensitivity to the deleterious mood and cognitive effects of lowered serotonin may represent an endophenotype for BD, by studying unaffected relatives of BD patients. In a double-blind, crossover design, 20 unaffected relatives from multiple bipolar families and 19 control subjects underwent acute tryptophan depletion (ATD) (25). Unlike the control subjects, unaffected relatives experienced a lowering of mood during ATD but not with the placebo. Furthermore, unaffected relatives tended to show increased impulsivity in the ATD condition. Measurements obtained before ingestion of the aminoacids drink indicated that, relative to control subjects, unaffected relatives exhibited lower serotonin platelet concentrations, lower affinity, and fewer binding sites of the serotonin transporter for imipramine; these differences were unaffected by tryptophan depletion. In more recent studies, Sobczak et al (26) investigated the effects of ATD on cognitive performance in healthy first-degree relatives of bipolar patients (FH) (N= 30) and matched controls (N= 15) in a placebo-controlled, double-blind crossover design. Performances on planning, memory and attention tasks were assessed at baseline and 5 hours after ATD. They found that speed of information processing on the planning task following ATD was impaired in the FH group but not in the control group. Furthermore, FH subjects with a bipolar disorder type I (BD-I) relative showed impairments in planning and memory, independent of ATD. In all subjects, ATD impaired long-term memory performance and speed of information processing. ATD did not affect short-term memory or focused and divided attention. Together, these results suggest that vulnerability to reduced tryptophan availability may represent an endophenotype for BD and warrants further investigation.

Studies assessing the sensitivity of the serotonergic system by exploring changes in plasma levels of prolactin and cortisol after administration of d-fenfluramine in manic patients have shown contradictory results (27,28). More consistent results have been found after administration of sumatriptan (a 5-HT_{1D} agonist): the growth hormone (GH) response is blunted in manic compared with depressed patients (29), revealing a subsensitivity of 5-HT function.

Dopaminergic system

Several lines of evidence point to a role of dopamine (DA) system in mood disorders. A relevant preclinical

model derives from the crucial role of mesoaccumbens DA in the neural circuitry of reward and/or incentive motivational behavior (30). Loss of motivation is one of the central features of depression and indeed anhedonia is one of the defining characteristics of melancholia. Thus, a deficiency of DA systems stands out as a prime candidate for involvement in the pathophysiology of depression (31,32). The strongest direct finding from clinical studies implicating DA in depression is reduced homovanillic acid (HVA, the major DA metabolite) in the CSF; indeed, this is one of the most consistent biochemical findings in depression (3,11). There is also evidence for a decreased rate of CSF HVA accumulation in subgroups of depressed patients, including those with marked psychomotor retardation versus agitation (33). Furthermore, depression occurs in up to 40% of patients with idiopathic Parkinson's disease and may precede motor symptoms. Interestingly, some case reports have documented abolition of symptoms of Parkinson's disease during a manic episode (34,35).

The pharmacological bridge also supports the notion that manipulation of the dopaminergic system is capable of modulating the illness. Thus, DA agonists appear to be effective antidepressants and are able to precipitate mania in some bipolar patients (3,11). Most recently, investigators have utilized a catecholamine depletion strategy (via use of the tyrosine hydroxylase inhibitor α -methylparatyrosine, AMPT) in lithium-treated, euthymic BD patients (36). Intriguingly, they did not observe any mood-lowering effects of AMPT, but observed a 'rebound hypomania' in a significant percentage of the patients. Although preliminary, these results are compatible with a dysregulated signaling system wherein the compensatory adaptation to catecholamine depletion results in an 'overshoot' due to impaired homeostatic mechanisms. Most recently, McTavish et al (37) reported that a tyrosine-free mixture lowered both subjective and objective measures of the psychostimulant effects of methamphetamine and manic scores. These preliminary studies suggest that tyrosine availability to the brain attenuates pathological increases in dopamine neurotransmission following methamphetamine administration and putatively in mania.

In more recent neuroimaging studies, the concentration of the vesicular monoamine transporter protein (VMAT2) was quantified with (+)[¹¹C]dihydrotetrabenazine (DTBZ) and PET (38). Sixteen asymptomatic BD-I patients who had a prior history of mania with psychosis (nine men and seven women) and individually matched healthy subjects were studied. VMAT2 binding in the thalamus and ventral brainstem of the bipolar patients was higher than in the comparison subjects. In a follow-up study, the same research group attempted to assess the diagnostic specificity of the findings, by comparing VMAT2 concentrations between euthymic BD-I (N=15) patients, schizophrenic patients (N=12), and age-matched healthy volunteers (N=15) (38). They found that VMAT2 binding in the thalamus was higher in BD-I patients than in control sub-

jects and schizophrenic patients. The authors interpreted the intriguing findings of increased VMAT2 expression in euthymic BD-I patients as representing trait-related abnormalities in the concentration of monoaminergic synaptic terminals. However, chronic lithium treatment has recently been demonstrated to increase VMAT2 protein in rat frontal cortex (the only region examined) (39), raising the possibility that the PET human studies may have been confounded by treatment effects.

Most recently, Yatham et al (40) assessed presynaptic dopamine function in 13 neuroleptic- and mood-stabilizer-naïve nonpsychotic first-episode manic patients by measuring [^{18}F]6-fluoro-L-DOPA (^{18}F -DOPA) uptake in the striatum by PET. No significant differences in ^{18}F -DOPA uptake rate constants in the striatum were found between the manic patients and the comparison subjects; however, treatment with valproate (VPA) significantly reduced ^{18}F -DOPA uptake rate.

Cholinergic system

Much of the evidence supporting the involvement of the cholinergic system in mood disorders comes from neurochemical, behavioral and physiologic studies in response to pharmacologic manipulations. These studies, carried out in the early 1970s, showed that the relative inferiority of noradrenergic compared to cholinergic tone was associated with depression, while the reverse was associated with mania (41). Additional support is found from a study on the central cholinesterase inhibitor physostigmine (administered intravenously), in which transient modulation of symptoms in manic cases and induction of depression in euthymic bipolar patients stabilized with lithium were observed.

A decrease in the cholinergic tone during mania has also been described when increased requirements of the cholinergic agonist pilocarpine were needed to elicit pupillary constriction: consistently, this responsiveness increased after lithium or VPA treatment (42,43), adding evidence on the effects of lithium perhaps potentiating brain cholinergic systems (44,45). However, the therapeutic responses observed with antidepressant and antimanic pharmacological agents are not reliably matched with effects on the cholinergic system.

Stress and glucocorticoids modulate neural plasticity: implications for mood disorders

Numerous reports document HPA axis hyperactivity in drug-free depressed (46) and bipolar depressed patients. With respect to BD, increased HPA activity has been associated with mixed manic states, depression, and less consistently with classical manic episodes (3,18). Chronic stress or glucocorticoid administration has been demonstrated to produce atrophy and death of vulnerable hippocampal neurons in rodents and primates. In humans,

magnetic resonance imaging (MRI) studies have also revealed reduced hippocampal volumes in patients with Cushing disease and post-traumatic stress disorder (other conditions associated with hypercortisolemia). Indeed, one of the most consistent effects of stress on cellular morphology is atrophy of the CA3 hippocampal neurons (47,48), which also occurs upon exposure to high levels of glucocorticoids, suggesting that activation of the HPA axis likely plays a major role in mediating the stress-induced atrophy (48). Thus, recurrent stress (and presumably recurrent mood episodes which are often associated with hypercortisolemia) may lower the threshold for cellular death/atrophy in response to a variety of physiological (e.g. aging) and pathological events, likely involving the inhibition of glucose transport (diminishing the capability for energy production and augmenting susceptibility to conditions which place a high demand or load on the neuron), and the abnormal enhancement of glutamatergic signaling leading to excitotoxicity (48).

SIGNALING NETWORKS: THE CELLULAR COGWHEELS UNDERLYING LONG-TERM NEUROPLASTICITY

More recently, research into the pathophysiology and treatment of mood disorders has moved from a focus on neurotransmitters and cell surface receptors to intracellular signaling cascades.

Multicomponent, cellular signaling pathways interact at various levels, thereby forming complex signaling networks which allow the cell to receive, process, and respond to information (49-51). These networks facilitate the integration of signals across multiple time scales, the generation of distinct outputs depending on input strength and duration, and regulate intricate feed-forward and feedback loops (49-51). Given their widespread and crucial role in the integration and fine-tuning of physiologic processes, it is not surprising that abnormalities in signaling pathways have now been identified in a variety of human diseases (2,52,53). Furthermore, signaling pathways represent major targets for a number of hormones, including glucocorticoids, thyroid hormones, and gonadal steroids (2,52). These biochemical effects may play a role in mediating certain clinical manifestations of altered hormonal levels in mood disorder subjects (e.g. the frequent onset of bipolar disorder in puberty, triggering of episodes in the postpartum period, association of depression and potentially rapid cycling with hypothyroidism, and triggering of affective episodes in response to exogenous glucocorticoids).

Complex signaling networks may be especially important in the CNS, where they 'weigh' and integrate diverse neuronal signals and then transmit these integrated signals to effectors, thereby forming the basis of a complex information processing network (49-51). The high degree of complexity generated by these signaling networks may be one mechanism by which neurons acquire the flexibility for generating the wide range of responses observed in the

nervous system. These pathways are thus undoubtedly involved in regulating such diverse vegetative functions as mood, appetite and wakefulness and are therefore likely to be involved in the pathophysiology of BD. We now turn to a discussion of the direct and indirect evidence supporting a role for abnormalities in signaling pathways in the pathophysiology and treatment of BD.

The Gs/cAMP generating signaling pathway

Several independent laboratories have now reported abnormalities in G protein subunits in BD (54,55). Post-mortem brain studies have reported increased levels of the stimulatory G protein (G_{α_s}) accompanied by increases in post-receptor stimulated adenylyl cyclase (AC) activity in BD (55,56). Several studies have also found elevated G_{α_s} protein levels and mRNA levels in peripheral circulating cells in BD, although the dependency on clinical state remains unclear (45,55,57-60). It should be emphasized, however, that there is at present no evidence to suggest that the alterations in the levels of G_{α_s} are due to a mutation in the G_{α_s} gene itself (61). There are numerous transcriptional and post-transcriptional mechanisms which regulate the levels of G protein subunits, and the elevated levels of G_{α_s} could potentially represent the indirect sequelae of alterations in any one of these other biochemical pathways (54,55,57,62).

There is growing consensus that the ability of a 'simple' monovalent cation like lithium to treat multiple aspects of an illness as complex as BD arises from its major effects on intracellular signaling pathways, rather than on any single neurotransmitter system per se (9,44,60). Although it appears that the lithium ion (at therapeutic concentrations) does not directly affect G protein function, there is considerable evidence that chronic lithium administration affects that function (9,44). Although some studies have reported modest changes in the levels of G protein subunits, the preponderance of the data suggests that chronic lithium does not modify G protein levels per se, but rather modifies G protein function (62,63). Although speculative, it might be postulated that these G protein effects - which would theoretically attenuate excessive signaling through multiple pathways - likely contribute to lithium's long-term prophylactic efficacy in protecting susceptible individuals from spontaneous-, stress-, and drug (e.g. anti-depressant, stimulant)- induced cyclic affective episodes.

The protein kinase C signaling pathway

Protein kinase C (PKC) exists as a family of closely related subspecies, has a heterogenous distribution in brain (with particularly high levels in presynaptic nerve terminals), and, together with other kinases, appears to play a crucial role in the regulation of synaptic plasticity and various forms of learning and memory (64-67). PKC is one of the major intracellular mediators of signals generated upon

external stimulation of cells via a variety of neurotransmitter receptors (including muscarinic M1, M3, M5 receptors, noradrenergic α_1 receptors, metabotropic glutamatergic receptors, and serotonergic 5-HT_{2A} receptors), which induce the hydrolysis of various membrane phospholipids.

To date, there have only been a limited number of studies directly examining PKC in BD (68). Although undoubtedly an over-simplification, particulate (membrane) PKC is sometimes viewed as the more active form of PKC, and thus an examination of the subcellular partitioning of this enzyme can be used as an index of the degree of activation. Friedman et al (69) investigated PKC activity and PKC translocation in response to serotonin in platelets obtained from BD subjects before and during lithium treatment. They reported that the ratios of platelet membrane-bound to cytosolic PKC activities were elevated in the manic subjects. In addition, serotonin-elicited platelet PKC translocation was found to be enhanced in those subjects. With respect to brain tissue, Wang and Friedman (70) measured PKC isozyme levels, activity and translocation in post-mortem brain tissue from BD patients; they reported increased PKC activity and translocation in BD brains compared to controls, effects which were accompanied by elevated levels of selected PKC isozymes in cortices of BD subjects.

Evidence accumulating from various laboratories has clearly demonstrated that lithium, at therapeutically relevant concentrations, exerts major effects on the PKC signaling cascade. Currently available data suggest that chronic lithium attenuates PKC activity, and downregulates the expression of PKC isozymes α and ϵ in frontal cortex and hippocampus (62,71). Chronic lithium has also been demonstrated to dramatically reduce the hippocampal levels of a major PKC substrate, MARCKS (myristoylated alanine rich C kinase substrate), which has been implicated in regulating long-term neuroplastic events (62,71). Although these effects of lithium on PKC isozymes and MARCKS are striking, a major problem inherent in neuropharmacologic research is the difficulty in attributing therapeutic relevance to any observed biochemical finding. It is thus noteworthy that the structurally dissimilar antimanic agent VPA produces very similar effects as lithium on PKC α and ϵ isozymes and MARCKS protein (63,71). Interestingly, lithium and VPA appear to bring about their effects on the PKC signaling pathway by distinct mechanisms. These biochemical observations are consistent with the clinical observations that some patients show preferential response to one or other of the agents, and that one often observes additive therapeutic effects in patients when the two agents are co-administered.

In view of the pivotal role of the PKC signaling pathway in the regulation of neuronal excitability, neurotransmitter release, and long-term synaptic events (68,72), it was postulated that the attenuation of PKC activity may play a role in the antimanic effects of lithium and VPA. In a pilot study it was found that tamoxifen (a non-steroidal antie-

strogen known to be a PKC inhibitor at higher concentrations (73)) may, indeed, possess antimanic efficacy (74). Clearly, these results have to be considered preliminary, due to the small sample size thus far. In view of the preliminary data suggesting the involvement of the PKC signaling system in the pathophysiology of BD, these results suggest that PKC inhibitors may be very useful agents in the treatment of mania. Larger double-blind placebo-controlled studies of tamoxifen and of novel selective PKC inhibitors in the treatment of mania are warranted.

Abnormalities of calcium signaling

Calcium ions play a critical role in regulating the synthesis and release of neurotransmitters, neuronal excitability, and long-term neuroplastic events, and it is thus not surprising that a number of studies have investigated intracellular Ca^{2+} in peripheral cells in BD (54,75). These studies have consistently revealed elevations in both resting and stimulated intracellular Ca^{2+} levels in platelets, lymphocytes and neutrophils of patients with BD. The regulation of free intracellular Ca^{2+} is a complex, multi-faceted process, and the abnormalities observed in BD could arise from abnormalities at a variety of levels (54). Ongoing studies should serve to delineate the specific regulatory sites at which the impairment occurs in BD.

IMPAIRMENTS OF NEUROPLASTICITY AND CELLULAR RESILIENCE

Structural imaging studies have demonstrated reduced gray matter volumes in areas of the orbital and medial PFC, ventral striatum and hippocampus, and enlargement of third ventricle in patients with mood disorders relative to healthy controls (76). Complementary post mortem neuropathological studies have shown abnormal reductions in cortex volume, glial cell counts, and/or neuronal densities/sizes in the subgenual PFC, orbital cortex and dorsal anterolateral PFC in unipolar and bipolar patients. However, many of these preliminary reports, although extremely interesting, require further replication.

The marked reduction in glial cells in these regions is particularly intriguing in view of the growing appreciation that glia plays critical roles in regulating synaptic glutamate concentrations and CNS energy homeostasis, and in releasing trophic factors that participate in the development and maintenance of synaptic networks formed by neuronal and glial processes (77). Abnormalities of glial function could thus prove integral to the impairments of structural plasticity and overall pathophysiology of mood disorders.

It is not presently known whether this evidence of neuronal deficits constitutes developmental abnormalities that may confer vulnerability to abnormal mood episodes, compensatory changes to other pathogenic processes, the sequelae of recurrent affective episodes or other factors that are difficult to control in patient populations.

Underlying mechanism for cell loss

Activation of the HPA axis appears to play a critical role in mediating hippocampal atrophy, as was already discussed. In addition to directly causing neuronal atrophy, stress and glucocorticoids also appear to reduce cellular resilience, thereby making certain neurons more vulnerable to other insults, such as ischemia, hypoglycemia, and excitatory aminoacid toxicity.

The reduction in the resilience of hippocampal neurons may also reflect the propensity for various stressors to decrease the expression of brain derived neurotrophic factor (BDNF) in this region (78). BDNF and other neurotrophic factors are necessary for the survival and function of neurons, implying that a sustained reduction of these factors could affect neuronal viability. Increasing evidence suggests that neurotrophic factors inhibit cell death cascades by (in large part) activating the mitogen activated protein (MAP) kinase signaling cascade, and upregulating major cell survival proteins such as bcl-2 (79). Bcl-2 is now recognized as a major neuroprotective protein, since bcl-2 overexpression protects neurons against diverse insults, including ischemia, the neurotoxic agent methyl-phenyl-tetrahydropyridine (MPTP), β -amyloid, free radicals, excessive glutamate, and growth factor deprivation (80). Accumulating data suggests that bcl-2 is not only neuroprotective, but also exerts neurotrophic effects and promotes neurite sprouting, neurite outgrowth and axonal regeneration (80). If enhanced bcl-2 expression appears to be capable of offsetting the potentially deleterious consequences of stress-induced neuronal endangerment (81), then, pharmacologically induced upregulation of bcl-2 may have considerable utility. Overall, it is clear that the neurotrophic factors/MAP kinase/bcl-2 signaling cascade plays a critical role in cell survival in the CNS, and that there is a fine balance maintained between the levels and activities of cell survival and cell death factors. Modest changes in this signaling cascade or in the levels of the bcl-2 family of proteins (potentially due to genetic, illness or insult-related factors) may therefore profoundly affect cellular viability.

Do antidepressants and mood stabilizers have neurotrophic properties?

'Neuroplasticity' subsumes diverse processes of vital importance by which the brain perceives, adapts and responds to a variety of internal and external stimuli. The manifestations of neuroplasticity in the adult CNS have been characterized as including alterations of dendritic function, synaptic remodeling, long-term potentiation (LTP), axonal sprouting, neurite extension, synaptogenesis, and even neurogenesis (82).

There are several reports supporting the hypothesis that antidepressant treatment produces neurotrophic-like effects (78). Chronic administration of an atypical antidepressant, tianeptine, was reported to block the stress-

induced atrophy of CA3 pyramidal neurons (83) and to block other stress-induced changes in brain structure and neurochemistry (84). Male tree shrews subjected to a chronic psychosocial stress paradigm were found to have decreased N-acetylaspartate (NAA), a putative marker of neuronal viability (85), measured in vivo by ¹H-magnetic resonance spectroscopy (MRS), decreased granule cell proliferation in the dentate gyrus of the hippocampus and a reduction in hippocampal volume as compared to non-stressed animals. All these stress-induced effects were prevented/reversed in the animals treated concomitantly with tianeptine (84). However, the generalizability of these effects to other classes of antidepressants is unclear.

Elegant recent studies have demonstrated that another pathway involved in cell survival and plasticity, the cyclic adenosine monophosphate (cAMP)-cAMP response element binding protein (CREB) cascade, is up-regulated by antidepressant treatment (86). Up-regulation of CREB, a gene promoter, and one of its major targets, BDNF, occurs in response to several different classes of antidepressant treatments, and occurs in a time frame consistent with the therapeutic action of antidepressants (87). Furthermore, chronic, but not acute, antidepressant treatments have been found to increase the number of new neurons in the dentate gyrus granule cell layer. These effects have been observed with different classes of antidepressants, but not with several other psychotropic medications investigated (87). A role for the cAMP-CREB cascade and BDNF in the actions of antidepressant treatment is also supported by studies demonstrating that up-regulation of these pathways has effects similar to antidepressant medications in behavioral models of depression such as the learned helplessness and forced swim test models (88-90).

Several endogenous growth factors, including nerve growth factor (NGF) and BDNF, exert many of their neurotrophic effects via the MAP kinase signaling cascade. The net result of stimulation of this cascade is an increase in the transcription and/or activity of a number of cell survival proteins, such as bcl-2 and BDNF. It is thus noteworthy that recent studies have demonstrated that chronic lithium and VPA robustly activate the MAP kinase cascade in cells of human neuronal origin and in rat frontal cortex and hippocampus (91,92). Consistent with this activation, both treatments produced a *doubling* of bcl-2 levels in frontal cortex, an effect primarily due to a marked increase in the number of bcl-2 immunoreactive cells in layers II and III of frontal cortex (93). Interestingly, the importance of neurons in layers II-IV of the frontal cortex in mood disorders has recently been emphasized, since primate studies indicate that these areas are important for providing connections with other cortical regions, and that they are targets for subcortical input (94). Furthermore, chronic lithium also increases bcl-2 levels in the mouse hippocampus (95) and in cerebellar granule cells in culture (96), as well as VPA increases bcl-2 levels in human cells of neuronal origin (91).

Consistent with the neurotrophic and neuroprotective effects of MAP kinase activation and bcl-2 upregulation, lithium, at therapeutically relevant concentrations, has been shown to exert neuroprotective effects in a variety of preclinical paradigms. Consistently, VPA has been demonstrated to exert neuroprotective actions in cellular models as well, including glutamate toxicity, β -amyloid toxicity, and following exposure to other toxins (97-100).

Glycogen synthase kinase: a common target for mood stabilizers

Lithium and VPA regulate the activity of a crucial kinase that functions as an intermediary in numerous intracellular signaling pathways, the enzyme glycogen synthase kinase-3 (GSK-3), suggesting the importance of this enzyme in BD research (101,102). While lithium inhibits GSK-3 - a constitutively active and a highly conserved enzyme in evolution - by direct competition with magnesium for a binding site, the precise mechanisms by which VPA exerts its action is still uncertain (102-104). Other signals deactivating GSK-3 arise from insulin stimulation, developmental signals, and numerous growth factors (e.g. NGF and BDNF). Thus, growth factors may bring about many of their neurotrophic/neuroprotective effects, at least in part, by GSK-3 inhibition. Rapidly increasing evidence suggests that GSK-3 also plays important roles in regulating neuroplasticity and cellular resilience. GSK-3 phosphorylates - and thereby inactivates - transcription factors and cytoskeletal proteins (such as the Alzheimer's protein tau). Furthermore, changes in GSK-3 mediate MAP-1B (a cytoskeletal protein) phosphorylation, associated with the loss and/or unbundling of stable axonal microtubules. Finally, GSK-3 β inhibition results in the accumulation of synapsin I, a protein involved in synaptic vesicle docking and release, at growth cone-like areas.

This evidence suggests that lithium's and VPA's effect on GSK-3 may play important roles in regulating processes such as synaptic plasticity and cell survival in the mature CNS. It is thus interesting that all of these processes have been implicated in the pathophysiology and treatment of BD (102).

Glutamatergic interventions: do they represent a neurotrophic strategy?

Another neurotransmitter system that has been implicated in regulating neuronal plasticity and cellular resilience in a variety of neuropsychiatric disorders is the highly complex glutamatergic system. In fact, glucocorticoids can induce the release of glutamate in the hippocampal CA3 region, and very high levels of type II corticosteroid receptor activation markedly increase calcium currents and lead to increased expression of N-methyl-D-aspartate (NMDA) receptor (a subtype of glutamatergic ionotropic receptor) on hippocampal neurons, that could predispose to neurotoxicity and finally atrophy. Interest-

ingly, NMDA blockade can prevent stress-induced atrophy in that region and it is thus noteworthy that recent preclinical studies have shown that the glutamatergic system represents a target (often indirect) for the actions of antidepressants and mood stabilizers (105).

Further evidence of the glutamatergic system involvement in mood disorders comes from brain imaging studies. These have shown that glucose metabolic signal, which correlates tightly with regional cerebral blood flow (CBF) during physiological activation, is likely to predominantly reflect glutamatergic transmission (106). PET imaging studies of BD patients have demonstrated abnormalities of CBF and glucose metabolism and, since projections from the regions involved in these abnormalities are glutamatergic, depression- and mania-related hypo/hyperactivity may be suggestive of either decreased (depression) or increased (mania) activation of glutamatergic cortico-limbic pathways. Thus, the hypothesis that a mood-stabilizing drug might modulate glutamate release or the consequences of glutamate release could be consistent with these data from functional neuroimaging studies.

There are a number of glutamatergic 'plasticity enhancing' strategies which may be of considerable utility in the treatment of mood disorders. Presently, lamotrigine and ketamine, two anti-glutamatergic agents, have shown to have antidepressant properties in bipolar and unipolar depression. Other agents - including NMDA antagonists, glutamate release reducing agents, and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors potentiators - are under development or currently being clinically tested (107).

Human evidence for the neurotrophic effects of mood stabilizers

While the body of preclinical data demonstrating neurotrophic and neuroprotective effects of mood stabilizers is striking, considerable caution must clearly be exercised in extrapolating to the clinical situation with humans. In view of lithium's and VPA's robust effects on the levels of the cytoprotective protein bcl-2 in the frontal cortex, Drevets et al re-analyzed older data demonstrating ~40% reductions in subgenual PFC volumes in familial mood disorder subjects (108). Consistent with neurotrophic/neuroprotective effects of lithium and VPA, they found that the patients treated with chronic lithium or VPA exhibited subgenual PFC volumes that were significantly higher than the volumes in non-treated patients, and not significantly different from controls (109). It should be noted that, in contrast to mood stabilizers, chronic treatment of patients with selective serotonin reuptake inhibitors did not have any effect on gray matter volumes. In a more recent study, Drevets and colleagues have investigated glial cell densities in mood disorder patients. Although the sample sizes are quite small, they made the intriguing observation that unipolar patients exhibited reduced glial cell densities,

whereas only the bipolar patients off chronic lithium or VPA exhibited similar reductions (110). Considerable caution is warranted in view of the small sample sizes and cross-sectional nature of the studies.

To investigate the potential neurotrophic effects of lithium in humans more definitively, a longitudinal clinical study was undertaken using proton MRS to quantitate NAA levels. It was found that chronic lithium increased NAA concentration in the human brain in vivo (111). These findings provide intriguing indirect support for the contention that chronic lithium increases neuronal viability/function in the human brain. Furthermore, a ~0.97 correlation between lithium-induced NAA increases and regional voxel gray matter content was observed, thereby providing evidence for co-localization with the regional specific bcl-2 increases observed in the rodent brain cortices. These results suggest that chronic lithium may not only exert robust neuroprotective effects (as it has been demonstrated in a variety of preclinical paradigms), but also exert neurotrophic effects in humans.

In follow-up studies to the NAA findings, it was hypothesized that, in addition to increasing functional neurochemical markers of neuronal viability, lithium-induced increases in bcl-2 would also lead to neuropil increases, and thus to increased brain gray matter volume in BD patients. In this clinical research investigation, brain tissue volumes were examined using high resolution three dimensional MRI and validated quantitative brain tissue segmentation methodology to identify and quantify the various components by volume, including total brain white and gray matter content. Measurements were made at baseline (medication free, after a minimum 14 day washout) and then repeated after 4 weeks of lithium at therapeutic doses. This study revealed that chronic lithium significantly increases total gray matter content in the human brain of patients with BD (112). No significant changes were observed in brain white matter volume, or in quantitative measures of regional cerebral water content, thereby providing strong evidence that the observed increases in gray matter content are likely due to neurotrophic effects as opposed to any possible cell swelling and/or osmotic effects associated with lithium treatment. A finer grained sub-regional analysis of this brain imaging data is ongoing. The increased gray matter finding has recently been replicated in a cross-sectional MRI study: Sassi et al (113) found that lithium treated bipolar patients had a statistically higher cortical gray matter volume when compared either to non-treated bipolar patients or control subjects.

CONCLUDING REMARKS

A considerable body of data confirms that the amine neurotransmitter systems are dysfunctional in BD, explaining why they have become a common target for pharmacological interventions. However, conceptual and

experimental evidence suggests that abnormalities in the regulation of signal transduction cascades and neuroplasticity could more primarily underlie the pathophysiology of BD. This concept is becoming increasingly important when considering that, for many refractory patients with this disorder, new drugs simply mimicking the 'traditional' medications which directly or indirectly alter neurotransmitter levels and those which bind to cell surface receptors may be of limited benefit (114). Therefore, the existence of abnormalities in signal transduction pathways suggests that, for patients refractory to conventional medications, improved therapeutics may only be obtained by the direct targeting of post-receptor sites (e.g. CREB/BDNF/MAP kinase/bcl-2). Strategies to enhance neurotrophic factor signaling are currently under research and they hold much promise for the development of novel therapeutics for the long-term treatment of severe BD.

References

- Weintraub B. Molecular endocrinology: basic concepts and clinical correlations. New York: Raven Press, 1995.
- Spiegel A. G proteins, receptors, and disease. Totowa: Humana Press, 1998.
- Goodwin FK, Jamison KR. Manic-depressive illness. New York: Oxford University Press, 1990.
- Bowden CL. Towards an integrated biological model of bipolar disorder. In: Young LT, Joffe RT (eds). Bipolar disorder: biological models and their clinical application. New York: Dekker, 1997:235-54.
- Wehr T, Goodwin F. Biological rhythms in manic-depressive illness. In: Wehr T, Goodwin F (eds). Circadian rhythms in psychiatry. Pacific Grove: Boxwood Press, 1983:129-84.
- Ikonomov OC, Manji HK. Molecular mechanisms underlying mood stabilization in manic-depressive illness: the phenotype challenge. *Am J Psychiatry* 1999;156:1506-14.
- Bunney W, Garland-Bunney B. Molecular clock genes in man and lower animals. Possible implications for circadian abnormalities in depression. *Neuropsychopharmacology* 2000;22:335-45.
- Holsboer F. Neuroendocrinology of mood disorders. In: Bloom FE, Kupfer DJ (eds). Psychopharmacology: the fourth generation of progress. New York: Raven Press, 1995:957-69.
- Manji HK, Lenox RH. The nature of bipolar disorder. *J Clin Psychiatry* 2000;61(Suppl. 13):42-57.
- Schatzberg AF, Schildkraut JJ. Recent studies on norepinephrine systems in mood disorders. In: Bloom FE, Kupfer DJ (eds). Psychopharmacology: the fourth generation of progress. New York: Raven Press, 1995:911-20.
- Manji H, Potter W. Monoaminergic mechanisms in bipolar disorder. In: Young LT, Joffe RT (eds). Bipolar disorder: biological models and their clinical application. New York: Dekker, 1997: 1-40.
- Young LT, Warsh JJ, Kish SJ et al. Reduced brain 5-HT and elevated NE turnover and metabolites in bipolar affective disorder. *Biol Psychiatry* 1994;35:121-7.
- Vawter MP, Freed WJ, Kleinman JE. Neuropathology of bipolar disorder. *Biol Psychiatry* 2000;48:486-504.
- Kirov G, Murphy KC, Arranz MJ et al. Low activity allele of catechol-O-methyltransferase gene associated with rapid cycling bipolar disorder. *Mol Psychiatry* 1998;3:342-5.
- Rotondo A, Mazzanti C, Dell'Osso L et al. Catechol-O-methyltransferase, serotonin transporter, and tryptophan hydroxylase gene polymorphisms in bipolar disorder patients with and without comorbid panic disorder. *Am J Psychiatry* 2002;159:23-9.
- Serretti A, Macciardi F, Verga M et al. Tyrosine hydroxylase gene associated with depressive symptomatology in mood disorder. *Am J Med Genet* 1998;81:127-30.
- Maes M, Meltzer H, D'Hondt P et al. Effects of serotonin precursors on the negative feedback effects of glucocorticoids on hypothalamic-pituitary-adrenal axis function in depression. *Psychoneuroendocrinology* 1995;20:149-67.
- Garlow S, Musselman D, Nemeroff C. The neurochemistry of mood disorders. In: Charney DS, Nester EJ, Bunney BS (eds). Neurobiology of mental illness. New York: Oxford University Press, 1999:348-64.
- Shiah IS, Yatham LN. Serotonin in mania and in the mechanism of action of mood stabilizers: a review of clinical studies. *Bipolar Disord* 2000;2:77-92.
- Drevets WC, Frank E, Price JC et al. PET imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry* 1999;46:1375-87.
- Sargent PA, Kjaer KH, Bench CJ et al. Brain serotonin 1A receptor binding measured by positron emission tomography with [¹¹C]WAY-100635: effects of depression and antidepressant treatment. *Arch Gen Psychiatry* 2000;57:174-80.
- Drevets WC, Frank E, Price JC et al. Serotonin type-1A receptor imaging in depression. *Nucl Med Biol* 2000; 27:499-507.
- Delgado PL, Miller HL, Salomon RM et al. Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. *Biol Psychiatry* 1999;46:212-20.
- Hughes JH, Dunne F, Young AH. Effects of acute tryptophan depletion on mood and suicidal ideation in bipolar patients symptomatically stable on lithium. *Br J Psychiatry* 2000;177:447-51.
- Quintin P, Benkelfat C, Launay JM et al. Clinical and neurochemical effect of acute tryptophan depletion in unaffected relatives of patients with bipolar affective disorder. *Biol Psychiatry* 2001;50:184-90.
- Sobczak S, Riedel WJ, Booij I et al. Cognition following acute tryptophan depletion: difference between first-degree relatives of bipolar disorder patients and matched healthy control volunteers. *Psychol Med* 2002;32:503-15.
- Thakore JH, O'Keane V, Dinan TG. d-Fenfluramine-induced prolactin responses in mania: evidence for serotonergic subsensitivity. *Am J Psychiatry* 1996;153:1460-3.
- Yatham LN. Prolactin and cortisol responses to fenfluramine challenge in mania. *Biol Psychiatry* 1996;39:285-8.
- Yatham LN, Zis AP, Lam RW et al. Sumatriptan-induced growth hormone release in patients with major depression, mania, and normal controls. *Neuropsychopharmacology* 1997;17:258-63.
- Wightman RM, Robinson DL. Transient changes in mesolimbic dopamine and their association with 'reward'. *J Neurochem* 2002;82:721-35.
- Fibiger HC. Dopaminergic-cholinergic interactions in the striatum. *Jpn J Psychiatry Neurol* 1991;45:512.
- Willner P, Muscat R, Phillips G. The role of dopamine in reward-related behavior: ability, insight, drive or incentive? *Pol J Pharmacol Pharm* 1991;43:291-300.
- Willner P, Scheel-Kruger J (eds). The mesolimbic dopamine system: from motivation to action. Chichester: Wiley, 1991.
- Larmande P, Palisson E, Saikali I et al. Disappearance of akinesia in Parkinson disease during a manic attack. *Rev Neurol* 1993;149:557-8.
- Scappa S, Teverbaugh P, Ananth J. Episodic tardive dyskinesia and parkinsonism in bipolar disorder patients. *Can J Psychiatry* 1993;38:633-4.
- Anand A, Darnell A, Miller HL et al. Effect of catecholamine depletion on lithium-induced long-term remission of bipolar disorder. *Biol Psychiatry* 1999;45:972-8.
- McTavish SF, McPherson MH, Harmer CJ et al. Antidopaminergic effects of dietary tyrosine depletion in healthy subjects and patients with manic illness. *Br J Psychiatry* 2001;179:356-60.

38. Zubieta JK, Huguelet P, Ohl LE et al. High vesicular monoamine transporter binding in asymptomatic bipolar I disorder: sex differences and cognitive correlates. *Am J Psychiatry* 2000;157:1619-28.
39. Zucker M, Weizman A, Harel D et al. Changes in vesicular monoamine transporter (VMAT2) and synaptophysin in rat substantia nigra and prefrontal cortex induced by psychotropic drugs. *Neuropsychobiology* 2001;44:187-91.
40. Yatham LN, Liddle PF, Shiah IS et al. PET study of [(18)F]6-fluoro-L-dopa uptake in neuroleptic- and mood-stabilizer-naïve first-episode nonpsychotic mania: effects of treatment with divalproex sodium. *Am J Psychiatry* 2002;159:768-74.
41. Janowsky DS, Overstreet DH. The role of acetylcholine mechanisms in mood disorders. In: Bloom FE, Kupfer DJ (eds). *Psychopharmacology: the fourth generation of progress*. New York: Raven Press, 1995:945-56.
42. Sokolski KN, DeMet EM. Pupillary cholinergic sensitivity to pilocarpine increases in manic lithium responders. *Biol Psychiatry* 1999;45:1580-4.
43. Sokolski KN, DeMet EM. Cholinergic sensitivity predicts severity of mania. *Psychiatry Res* 2000;95:195-200.
44. Jope RS. Anti-bipolar therapy: mechanism of action of lithium. *Mol Psychiatry* 1999;4:117-28.
45. Lenox R, Manji H. Drugs for treatment of bipolar disorder: lithium. In: Schatzberg AF, Nemeroff CB (eds). *Textbook of psychopharmacology*, 2nd ed. Washington: American Psychiatric Press, 1998:379-429.
46. Holsboer F. Stress, hypercortisolism and corticosteroid receptors in depression: implications for therapy. *J Affect Disord* 2001;62:77-91.
47. McEwen BS. Stress and hippocampal plasticity. *Annu Rev Neurosci* 1999;22:105-22.
48. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry* 2000;57:925-35.
49. Bourne HR, Nicoll R. Molecular machines integrate coincident synaptic signals. *Cell* 1993;72(Suppl.):65-75.
50. Bhalla US, Iyengar R. Emergent properties of networks of biological signaling pathways. *Science* 1999;283:381-7.
51. Weng G, Bhalla US, Iyengar R. Complexity in biological signaling systems. *Science* 1999;284:92-6.
52. Manji HK. G proteins: implications for psychiatry. *Am J Psychiatry* 1992;149:746-60.
53. Milligan G, Wakelam M. G proteins: signal transduction and disease. San Diego: Academic Press, 1992.
54. Li P, Andreopoulos S, Warsh J. Signal transduction abnormalities in bipolar affective disorder. In: Reith MEA (ed). *Cerebral signal transduction*. Totowa: Humana Press, 2000:283-312.
55. Warsh J, Young L, Li P. Guanine nucleotide binding (G) protein disturbances. In: Manji H, Bowden C, Belmaker R (eds). *Bipolar medications: mechanisms of action*. Washington: American Psychiatric Press, 2000:299-329.
56. Young LT, Li PP, Kish SJ et al. Cerebral cortex Gs alpha protein levels and forskolin-stimulated cyclic AMP formation are increased in bipolar affective disorder. *J Neurochem* 1993;61:890-8.
57. Manji HK, Chen G, Shimon H et al. Guanine nucleotide-binding proteins in bipolar affective disorder. Effects of long-term lithium treatment. *Arch Gen Psychiatry* 1995;52:135-44.
58. Mitchell PB, Manji HK, Chen G et al. High levels of Gs alpha in platelets of euthymic patients with bipolar affective disorder. *Am J Psychiatry* 1997;154:218-23.
59. Spleiss O, van Calker D, Scharer L et al. Abnormal G protein alpha(s)- and alpha(i2)-subunit mRNA expression in bipolar affective disorder. *Mol Psychiatry* 1998;3:512-20.
60. Wang HY, Friedman E. Effects of lithium on receptor-mediated activation of G proteins in rat brain cortical membranes. *Neuropharmacology* 1999;38:403-14.
61. Ram A, Guedj F, Cravchik A et al. No abnormality in the gene for the G protein stimulatory alpha subunit in patients with bipolar disorder. *Arch Gen Psychiatry* 1997;54:44-8.
62. Manji HK, Lenox RH. Signaling: cellular insights into the pathophysiology of bipolar disorder. *Biol Psychiatry* 2000;48: 518-30.
63. Manji H, Chen G, Hsiao J et al. Regulation of signal transduction pathways by mood stabilizing agents: implications for the pathophysiology and treatment of bipolar affective disorder. In: Manji H, Bowden C, Belmaker R (eds). *Bipolar medications: mechanisms of action*. Washington: American Psychiatric Press, 2000:129-77.
64. Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* 1992;258:607-14.
65. Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. *Faseb J* 1995;9:484-96.
66. Stabel S, Parker PJ. Protein kinase C. *Pharmacol Ther* 1991; 51:71-95.
67. Newton AC. Protein kinase C. Structure, function, and regulation. *J Biol Chem* 1995;270:28495-8.
68. Hahn CG, Friedman E. Abnormalities in protein kinase C signaling and the pathophysiology of bipolar disorder. *Bipolar Disord* 1999;1:81-6.
69. Friedman E, Hoau Yan W, Levinson D et al. Altered platelet protein kinase C activity in bipolar affective disorder, manic episode. *Biol Psychiatry* 1993;33:520-5.
70. Wang HY, Friedman E. Enhanced protein kinase C activity and translocation in bipolar affective disorder brains. *Biol Psychiatry* 1996;40:568-75.
71. Manji HK, Lenox RH. Protein kinase C signaling in the brain: molecular transduction of mood stabilization in the treatment of manic-depressive illness. *Biol Psychiatry* 1999;46:1328-51.
72. Conn P, Sweatt J. Protein kinase C in the nervous system. In: Kuo J (ed). *Protein kinase C*. New York: Oxford University Press, 1994:199-235.
73. Couldwell WT, Weiss MH, DeGiorgio CM et al. Clinical and radiographic response in a minority of patients with recurrent malignant gliomas treated with high-dose tamoxifen. *Neurosurgery* 1993;32:485-9.
74. Bechuk JM, Arfken CL, Dolan-Manji S et al. A preliminary investigation of a protein kinase C inhibitor in the treatment of acute mania. *Arch Gen Psychiatry* 2000;57:95-7.
75. Dubovsky SL, Murphy J, Thomas M et al. Abnormal intracellular calcium ion concentration in platelets and lymphocytes of bipolar patients. *Am J Psychiatry* 1992;149:118-20.
76. Drevets WC. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Curr Opin Neurobiol* 2001;11:240-9.
77. Coyle JT, Schwarcz R. Mind glue: implications of glial cell biology for psychiatry. *Arch Gen Psychiatry* 2000;57:90-3.
78. Duman RS. Synaptic plasticity and mood disorders. *Mol Psychiatry* 2002;7(Suppl. 1):S29-34.
79. Manji HK, Chen G. PKC, MAP kinases and the bcl-2 family of proteins as long-term targets for mood stabilizers. *Mol Psychiatry* 2002;7(Suppl. 1):S46-56.
80. Manji HK, Moore GJ, Chen G. Lithium up-regulates the cytoprotective protein Bcl-2 in the CNS in vivo: a role for neurotrophic and neuroprotective effects in manic depressive illness. *J Clin Psychiatry* 2000;61(Suppl. 9):82-96.
81. Manji HK, Quiroz JA, Gould TD. Cellular resilience and neuroplasticity in mood disorders. *Psychiatric Times* 2003(Jan): 55-9.
82. Mesulam MM. Neuroplasticity failure in Alzheimer's disease: bridging the gap between plaques and tangles. *Neuron* 1999;24:521-9.
83. Watanabe Y, Gould E, Daniels DC et al. Tianeptine attenuates stress-induced morphological changes in the hippocampus. *Eur J Pharmacol* 1992;222:157-62.
84. Czeh B, Michaelis T, Watanabe T et al. Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. *Proc Natl Acad Sci USA* 2001;98:12796-801.
85. Tsai G, Coyle JT. N-acetylaspartate in neuropsychiatric disorders. *Prog Neurobiol* 1995;46:531-40.

86. Thome J, Sakai N, Shin K et al. cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J Neurosci* 2000;20:4030-6.
87. Malberg JE, Eisch AJ, Nestler EJ et al. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 2000;20:9104-10.
88. Siuciak JA, Lewis DR, Wiegand SJ et al. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol Biochem Behav* 1997;56:131-7.
89. Chen AC, Shirayama Y, Shin KH et al. Expression of the cAMP response element binding protein (CREB) in hippocampus produces an antidepressant effect. *Biol Psychiatry* 2001;49:753-62.
90. Shirayama Y, Chen AC, Nakagawa S et al. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* 2002;22:3251-61.
91. Yuan PX, Huang LD, Jiang YM et al. The mood stabilizer valproic acid activates mitogen-activated protein kinases and promotes neurite growth. *J Biol Chem* 2001;276:31674-83.
92. Chen G, Einat H, Yuan P et al. Evidence for the involvement of the MAP/ERK signaling pathway in mood modulation. *Biol Psychiatry* 2002;51:126S.
93. Chen G, Zeng WZ, Yuan PX et al. The mood-stabilizing agents lithium and valproate robustly increase the levels of the neuroprotective protein bcl-2 in the CNS. *J Neurochem* 1999;72:879-82.
94. Rajkowska G, Miguel-Hidalgo JJ, Wei J et al. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry* 1999;45:1085-98.
95. Chen G, Rajkowska G, Du F et al. Enhancement of hippocampal neurogenesis by lithium. *J Neurochem* 2000;75:1729-34.
96. Chen RW, Chuang DM. Long term lithium treatment suppresses p53 and Bax expression but increases Bcl-2 expression. A prominent role in neuroprotection against excitotoxicity. *J Biol Chem* 1999;274:6039-42.
97. Bruno V, Sortino MA, Scapagnini U et al. Antidegenerative effects of Mg(2+)-valproate in cultured cerebellar neurons. *Funct Neurol* 1995;10:121-30.
98. Mark RJ, Ashford JW, Goodman Y et al. Anticonvulsants attenuate amyloid beta-peptide neurotoxicity, Ca²⁺ deregulation, and cytoskeletal pathology. *Neurobiol Aging* 1995;16:187-98.
99. Manji HK, Moore GJ, Chen G. Clinical and preclinical evidence for the neurotrophic effects of mood stabilizers: implications for the pathophysiology and treatment of manic-depressive illness. *Biol Psychiatry* 2000;48:740-54.
100. Hashimoto R, Hough C, Nakazawa T et al. Lithium protection against glutamate excitotoxicity in rat cerebral cortical neurons: involvement of NMDA receptor inhibition possibly by decreasing NR2B tyrosine phosphorylation. *J Neurochem* 2002;80:589-97.
101. Chen G, Huang LD, Jiang YM et al. The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. *J Neurochem* 1999;72:1327-30.
102. Gould TD, Manji HK. The wnt signaling pathway in bipolar disorder. *Neuroscientist* 2002;8:187-201.
103. Phiel CJ, Klein PS. Molecular targets of lithium action. *Annu Rev Pharmacol Toxicol* 2001;41:789-813.
104. Klein PS, Melton DA. A molecular mechanism for the effect of lithium on development. *Proc Natl Acad Sci USA* 1996;93:8455-9.
105. Skolnick P, Layer RT, Popik P et al. Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. *Pharmacopsychiatry* 1996;29:23-6.
106. Magistretti PJ, Pellerin L. Cellular mechanisms of brain energy metabolism. Relevance to functional brain imaging and to neurodegenerative disorders. *Ann N Y Acad Sci* 1996;777:380-7.
107. Zarate C, Quiroz J, Payne J et al. Modulators of the glutamatergic system: implications for the development of improved therapeutics in mood disorders. *Psychopharmacol Bull* 2003;36:35-83.
108. Drevets WC, Price JL, Simpson JR Jr et al. Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 1997;386:824-7.
109. Drevets WC. Neuroimaging studies of mood disorders. *Biol Psychiatry* 2000;48:813-29.
110. Bowley MP, Drevets WC, Ongur D et al. Low glial numbers in the amygdala in major depressive disorder. *Biol Psychiatry* 2002;52:404-12.
111. Moore GJ, Bechuk JM, Hasanat K et al. Lithium increases N-acetyl-aspartate in the human brain: in vivo evidence in support of bcl-2's neurotrophic effects? *Biol Psychiatry* 2000;48:1-8.
112. Moore GJ, Bechuk JM, Wilds IB et al. Lithium-induced increase in human brain grey matter. *Lancet* 2000;356:1241-2.
113. Sassi R, Nicoletti M, Brambilla P et al. Increased gray matter volume in lithium-treated bipolar disorder patients. *Neurosci Lett* 2002;329:243.
114. Nestler EJ. Antidepressant treatments in the 21st century. *Biol Psychiatry* 1998;44:526-33.